

QIAGEN Supplementary Protocol

Use of the EZ1[®] Advanced XL Flip Cap Rack

This document describes the protocols for automated purification of mitochondrial and total genomic DNA from forensic samples using the Flip Cap Rack and the EZ1 Advanced XL Flip Cap Card. The protocols use the EZ1 DNA Investigator[®] Kit on the EZ1 Advanced XL (up to 14 samples per run) with various forensic sample types. The Flip Cap Rack enables use of 2 ml flip cap tubes as sample tubes and 1.5 ml flip cap tubes as elution tubes as well as screw cap sample and elution tubes provided with the EZ1 DNA Investigator Kit.

IMPORTANT: Please see the *EZ1 DNA Investigator Kit Handbook* for general information on handling and storage of kit components. Please see the *EZ1 Advanced XL User Manual* for detailed information about instrument setup. Do not use the Flip Cap Rack in combination with protocol cards other than the EZ1 Advanced XL Flip Cap Card. Do not use flip cap tubes other than those stated in "Equipment and Reagents".

Equipment and reagents

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- EZ1 DNA Investigator Kit (48) (cat.no. 952034)
- EZ1 Advanced XL instrument (cat. no. 9001492)
- EZ1 Advanced XL Flip Cap Card (cat. no. 9022763)
- EZ1 Advanced XL Flip Cap Rack (cat. no. 9022818)
- One of the following 2 ml sample tubes:
 - Eppendorf[®] Safe Lock 2 ml (Eppendorf, cat. no. 0030 120.094)
 - Sarstedt SafeSeal 2 ml (Sarstedt, cat. no. 72.695.500)
 - Brand Microcentrifuge Tube 2 ml (Brand, cat. no. 780550)
 - Sarstedt 2 ml EZ1 screw-cap sample tube (Sarstedt, cat. no. 72-694.406)
 - Sample Tubes RB 2 ml (QIAGEN QIAcube[®] accessories, cat. no. 990381)
- One of the following 1.5 ml elution tubes:
 - Eppendorf 1.5 ml 3810X (Eppendorf, cat. no. 0030 125.150)
 - Eppendorf Safe Lock 1.5 ml 3810X (Eppendorf, cat. no. 0030 120.086)
 - Sarstedt 1.5 ml (Sarstedt, cat. no. 72.690.001)
 - Sarstedt 1.5 ml EZ1 screw-cap elution tube (Sarstedt, cat. no. 72-692.405)



Important points before starting

- Lyse the samples according to the corresponding pretreatment protocols described in the *EZ1 DNA Investigator Kit Handbook*.
- All steps of the protocol must be performed at room temperature (15–25°C), so work quickly during the setup procedure.
- The reagent cartridges and Buffer MTL contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, they must be equilibrated to operating temperature before use. Place the reagent cartridge into a shaker–incubator and incubate at 30–40°C with gentle agitation for at least 2 hours before use. If precipitates are visible at the bottom of the wells, redissolve by incubating at 30–40°C with gentle agitation for a further 2 hours. Do not use the reagent cartridges if the precipitates do not redissolve.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve buffer using gentle agitation at 37°C, and then place at room temperature (15–25°C).

Procedure

1. **Insert the EZ1 Advanced XL DNA Investigator Flip Cap Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL.**
2. **Switch on the EZ1 Advanced XL instrument.**
3. **Press “START” to start protocol setup. Follow the onscreen instructions for data tracking.**
4. **Press “1” (for Trace protocol), “2” (for Trace TD protocol), “3” (for Large-Volume protocol), “4” (for Norm protocol), or “5” (for Norm TD protocol).**
5. **Choose the elution buffer and volume: press “1” to elute into water or “2” to elute into TE buffer. Then press “1”, “2”, “3”, or “4” to select the elution volume.**
6. **Press any key to proceed through the text shown on the display and start worktable setup.**
7. **Open the instrument door.**
8. **Invert reagent cartridges twice to mix the magnetic particles. Tap the cartridges to deposit the reagents at the bottom of their wells. Check to see that the magnetic particles have been completely resuspended.**
9. **Load the reagent cartridges into the cartridge rack.**
Note: After sliding a reagent cartridge into the cartridge rack, press down on the cartridge until it clicks into place.
10. **Load opened elution tubes into the first row of the tip rack.**
Note: Ensure that tubes and caps are properly inserted if using flip cap tubes.

11. **Load tip holders containing filter-tips into the second row of the tip rack.**
12. **Load opened sample tubes containing digested samples into the back row of the tip rack.**

Note: Ensure that tubes and caps are properly inserted if using flip cap tubes.

Note: When using the data tracking option, ensure that the sample ID follows the same order as the samples on the worktable to avoid data mixup.

Note: If samples are to be processed with the Large-Volume protocol, add 400 μ l Buffer MTL to each sample tube containing digested samples.

13. **Close the instrument door.**
14. **Press "START" to start the purification procedure.**
The automated purification procedure takes 15 - 20 min.
15. **When the protocol ends, the display shows "Protocol finished". Press "ENT" to generate the report file.**

The EZ1 Advanced XL can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.

16. **Open the instrument door.**
17. **Retrieve the elution tubes containing the purified DNA. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods. Discard the sample-preparation waste.***

If the purified DNA is to be analyzed by real-time PCR, tubes containing eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube in order to minimize the risk of magnetic-particle carryover.

18. **Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.**
19. **To run another protocol, press "ESC", prepare samples as described in "Pretreatment protocol", and follow the procedure from step 4 onward. Otherwise, press "STOP" twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 Advanced XL instrument.**
20. **Clean the EZ1 Advanced XL instrument.**

Follow the maintenance instructions in the user manual supplied with your EZ1 Advanced XL instrument.

* Sample waste contains guanidine salts and is therefore not compatible with bleach.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Selected handbooks can be downloaded from www.qiagen.com/literature. Safety data sheets (SDS) for any QIAGEN product can be downloaded from www.qiagen.com/safety.

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